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Stomach Specific Drug Delivery System of Ketoconazole Floating Alginate Beads

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Keywords: Ketoconazole, Sodium Alginate, HPMC K15M, Olive Oil, Central Composite Design, UV-Spectrophotometer, Short-term stability.

ABSTRACT

In the present research work, a multiple unit-type oral floating dosage form of Ketoconazole capable of floating in the gastric condition were developed using an ionotropic gelation method to prolong gastric residence time. Ketoconazole is an antifungal drug which is used in the treatment of fungal infections. In this study, an attempt was made to increase the gastric residence time of Ketoconazole in acidic pH. Preformulation study was carried out by performing an identification test, a solubility test, and compatibility study. Central composite design model was used to study different formulations. The formulations of stomach-specific floating alginate beads were formulated by using polymers sodium alginate and HPMC K15M, Olive oil was used as a floating agent and they were evaluated by using micrometric and physical parameters which include measurement of bulk density, tapped density, Carr's index, angle of repose, practical yield, particle size, entrapment efficiency, swelling index, floating time and Surface morphology. Drug content was estimated by using UV-Spectrophotometric method and results were found to be within the acceptance limit. The release profile of the drug was determined by *in vitro* dissolution study. Short term stability studies also performed and results revealed that formulation was found to be stable and effective.

INTRODUCTION

Ketoconazole is a synthetic, broad-spectrum antifungal agent containing imidazole as a basic heterocyclic nucleus. The oral absorption of Ketoconazole is facilitated by gastric acidity, being a weak acid the drug is well absorbed from the upper portion of the duodenum. Ketoconazole is a weak dibasic compound that has pKa values of 6.5 and 2.9. It shows a pH-dependent solubility profile. Ketoconazole has an excellent solubility at acidic pH below 3, but its solubility drops dramatically to <1 mg/ml at pH 4. The solubility of Ketoconazole decreases to only 0.002 mg/ml with an increase in pH. Ketoconazole shows high solubility at acidic pH results in its complete dissolution in normal stomach. However, as it moves into the higher pH (pH 6–7) environment of a small intestine, precipitation of Ketoconazole is a concern. In addition, at the higher pH conditions of a hypochlorhydric stomach, Ketoconazole incompletely dissolves, resulting in diminished bioavailability. As oral absorption of Ketoconazole is facilitated by gastric acidity, it was thought to formulate floating alginate beads of Ketoconazole to increase its solubility in the gastric pH [1,2]. Gastroretentive drug delivery systems are the systems which are retained in the stomach for a longer period of time and thereby, improve the bioavailability of drugs. Gastric retention can be achieved by the mechanism of mucoadhesion, floatation, swelling, high density, sedimentation. Floating microbeads improve patient compliance by decreasing dosing frequency, the better therapeutic effect of short half-life drugs can be achieved [3].In the present study, an attempt has been made to prepare floating alginate beads of ketoconazole by ionotropic gelation method to increase gastric residence time in acidic pH.

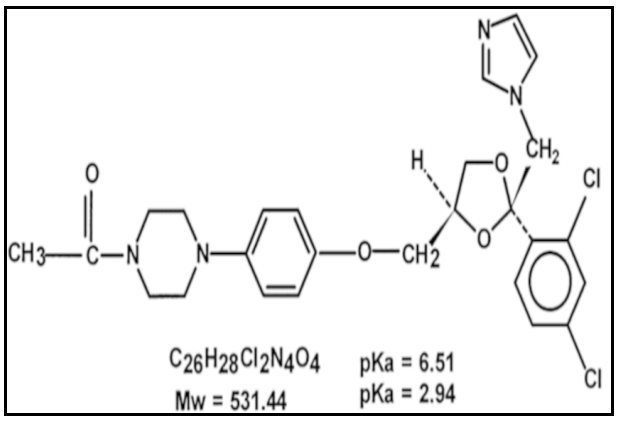


Figure no 1: Chemical structure of Ketoconazole

MATERIALS AND METHODS

Instruments used:

Electronic analytical balance, UV-Spectrophotometer, Differential Scanning Colorimeter (DSC), FTIR Spectrophotometer, Scanning Electron Microscope (SEM), Dissolution tester.

Chemicals and Reagents:

Hydrochloric acid, Sodium hydroxide, HPMC K15M, Sodium alginate, Olive oil. All chemicals used were pure and analytical grade.

Drug, Polymers and Additives Details:

Ketoconazole was obtained as a gift sample from Cipla Pvt. Ltd., Goa. HPMC K15M was obtained as a gift sample from Colorcon Pvt. Ltd., Verna Goa. Sodium alginate was obtained as a gift sample from Bombay Research Laboratory, Poona. Olive oil was obtained as a gift sample from Venkatrammana industry UP, India.

Preformulation studies [4]:

Preformulation study of Ketoconazole includes identification tests, solubility analysis, and compatibility study. Identification of drug was performed by FTIR Study, Solubility analysis was performed by utilizing different solvents and compatibility studies were performed by using FTIR and Differential Scanning Colorimetric analysis. The FTIR spectrum of the sample drug was obtained and compared with the standard FTIR spectrum of the pure drug. Preformulation solubility study was carried out in different solvents to check the suitable solvent system for the drug as well as various excipients used for the formulation and also to test drug solubility in the dissolution medium. FTIR spectrum of Ketoconazole and a physical mixture of Ketoconazole with Sodium alginate and HPMC K15M were recorded by using FTIR (affinity-1, Shimadzu, Kyoto, Japan) in order to determine the physical and chemical interactions between the drug and the excipients. The DSC thermograms of ketoconazole and a physical mixture of ketoconazole with polymers were recorded by using DSC. The purpose of this study is to determine the compatibility of drug and the excipients.

Standard Calibration Curve of Ketoconazole in 0.1N HCl buffer:

100 mg of Ketoconazole was transferred in 100 ml volumetric flask containing 0.1N HCl buffer. The stock solution was serially diluted with 0.1N HCl to get drug concentration in the Beer's range of 0-14 µg/ml. The absorbance of the solutions was measured against 0.1N HCl as a blank at 224.5nm using UV visible spectrophotometer. The graph of absorbance/s concentration (µg/ml) was plotted [5].

Design of Experiment:

Central composite design set up using software Design Expert 9.0.6.2 consisting of 2 factors and 3 levels was used to study the effect of independent variables. Sodium alginate and Olive oil on the product quality attributes[6].

Formulation of stomach-specific floating alginate beads of Ketoconazole [7,8]:

Floating alginate beads were prepared by ionotropic gelation method. 200mg of Ketoconazole was first dissolved in methanol. Sodium alginate with different proportions (1.5%.2.15% and 2.85%) was dissolved in distilled water and HPMC K15M was kept constant in all 9 formulations (0.3%) was dissolved in distilled water. To the polymer solution, the prepared drug solution along with olive oil with different concentrations (7%, 10%, and 12%) was added and stirred on a magnetic stirrer to form a homogeneous solution. After stirring the drug-loaded polymeric solution was extruded through a 22G syringe needle into calcium chloride solution (5%w/v) maintained under gentle agitation on a magnetic stirrer. The formed beads were allowed to remain in the same solution for about 1 hour to increase hardness and to improve mechanical strength. The beads formed were filtered and kept overnight at room temperature for drying. 10 formulations are prepared using the software-generated amount of Sodium alginate and olive oil. The response variables particle size, floating time and *in vitro* dissolution time are obtained experimentally. Experimental values are compared with software generated predicted values. Response variables were subjected to one way ANOVA at 0.05.Based on optimization results, the software generates optimized solutions for independent variables $\mathbf{X_1}$ and $\mathbf{X_{2\ and}}$ also for response variables $\mathbf{Y_1}$, $\mathbf{Y_2}$, $\mathbf{Y_3}$. Finally, a checkpoint batch F10 (optimized formulation) using optimized values is prepared to prove the validity and evolved method.

Evaluation of floating microbeads[9,10,11]:

Prepared floating microbeads of Ketoconazole were evaluated by using micrometric and physical parameters. Micrometric parameters were determined by measuring bulk density, tapped density, Carr's index, and angle of repose. Physical properties of floating beads were determined by measuring practical yield, particle size, entrapment efficiency, swelling index, floating time and Surface morphology.

- **The practical yield** was calculated as the weight of beads recovered from each batch in relation to the sum of the starting material. It was determined by using the formula**:**%Yeild= Weight of the beads/ Weight of drug +Polymer weight × 100.

- **Particle size** was determined using the optical microscopic method with the help of ocular and stage micrometer. Measurement of each sample was repeated three times and standard deviations were recorded.

- **Entrapment efficiency** was measured as follows, 100 mg of dried beads were placed into 100 ml of 0.1 N HCl. It was shaken for 6 hours and the resulting solution was filtered. The filtrate was suitably diluted and analyzed at 224nm UV spectrophotometer. It was calculated by using the following equation**:**

$$\%\ \text{entrapment efficiency} = \frac{\text{Practical drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

- **Swelling index** was measured as follows,50 mg of floated beads was soaked in 900ml 0f buffer (0.1N HCl) at 37± 0.5ºC. Then the beads were removed at specified time intervals, dried and weighed to determine the swelling index, swelling ratio was determined from the following equation:% Swelling index = W2-W1/W1 × 100**(**W1 is the weight of dried beads and W2 - the weight of swollen beads).

- **Floating time of beads:** 50mg of floated beads was placed in a beaker containing 100ml of 0.1N HCl buffer. The number of floating beads on the surface was evaluated at fixed time intervals. The floating time was considered as the time at which the 100% of the beads floated.

➢ **Surface morphology:** a Morphological examination of the surface of the dried beads were carried out by using scanning electron microscope (JEOI-SEM 6360; Japan at a magnification of 500X & 50X) at a voltage of 20 kV.

Estimation of drug content:

Weighed 100 mg of Ketoconazole floating beads and dissolved in 100ml of 0.1N HCl sonicated for half hour. This solution was kept overnight for the complete dissolution of the Ketoconazole in 0.1N HCl. This solution was filtered and further diluted to make a concentration of 10µg/ml solution. The absorbance of the solutions was measured at 224.5nm using double-beam UV against 0.1N HCl solution as the blank.

***In Vitro* drug release profile:**

In vitro release profile of beads was evaluated employing USP XXIII dissolution apparatus type 1. The dissolution test was performed using 900ml of 0.1N HCl as dissolution medium maintained at 37±0.5ºC. Aliquots of 1ml of the sample were withdrawn at predetermined intervals from the receptor compartment and the same was replaced with fresh buffer. The drug release was determined spectrophotometrically after dilution by measuring the absorbance at 224.5 nm (UV Shimadzu UV-1700).

Kinetic study of drug release profile:

To analyze the mechanism of drug release and release rate kinetics of the dosage form, the data obtained were fitted into zero order, first order, Higuchi matrix, and Korsmeyer-Peppas model. Based on the R^2 value best fit model was selected.

Stability study[12]:

Beads were kept in a tightly closed glass container and subjected to short-term and accelerated stability studies. The weighed quantity of the samples were exposed to short-term stability conditions at 25°C±2°C/60%RH±5%RHand accelerated stability studies were carried out at 40°C±2°C/ 75%RH±5%RH for a period of 1 months in humidity control oven (Lab Control, Ajinkya IM 3500 Series, India). After 15 days and 30 days, the samples were taken out and analyzed for drug content, density, floating, floating lag time and *in vitro* drug release.

RESULTS

Preformulation studies:

Ketoconazole was obtained and identified by FTIR analysis. IR spectrum of the pure drug was found to be similar to the reference standard IR spectrum of Ketoconazole Figure1. Solubility analysis showed that Ketoconazole was found to be soluble in olive oil (20.84mg/ml) and 0.1 N HCl buffer (18.81mg/ml), freely soluble in methanol (20.33mg/ml). The IR spectra revealed that there was no interaction between the drug and the polymer figures 2. The DSC thermograms of pure Ketoconazole showed a sharp endotherm peak ay 150.9°C corresponding to its melting point. Hence by studying FTIR and DSC spectrums of drug and polymer, it was observed that there was no incompatibility. The mixture of drug and polymers were compatible with Figure 4.

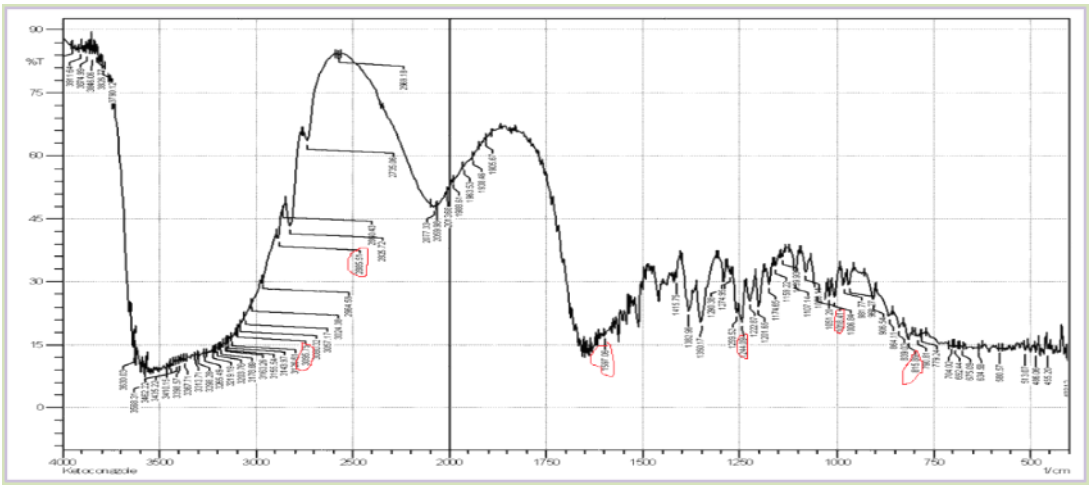


Fig. (2): FTIR spectrum of pure drug Ketoconazole

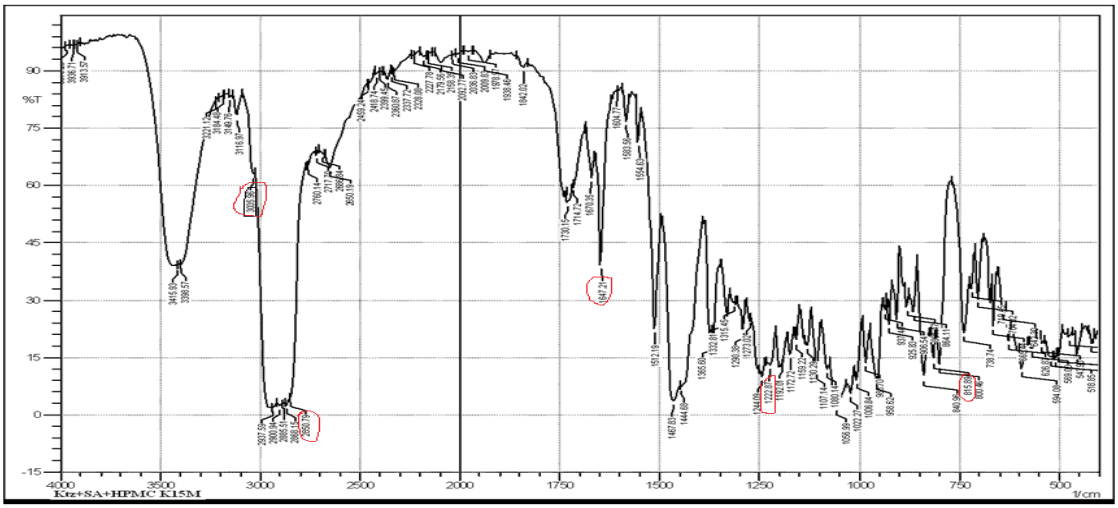


Fig. (3): FTIR spectrum of a physical mixture of Ketoconazole and polymers

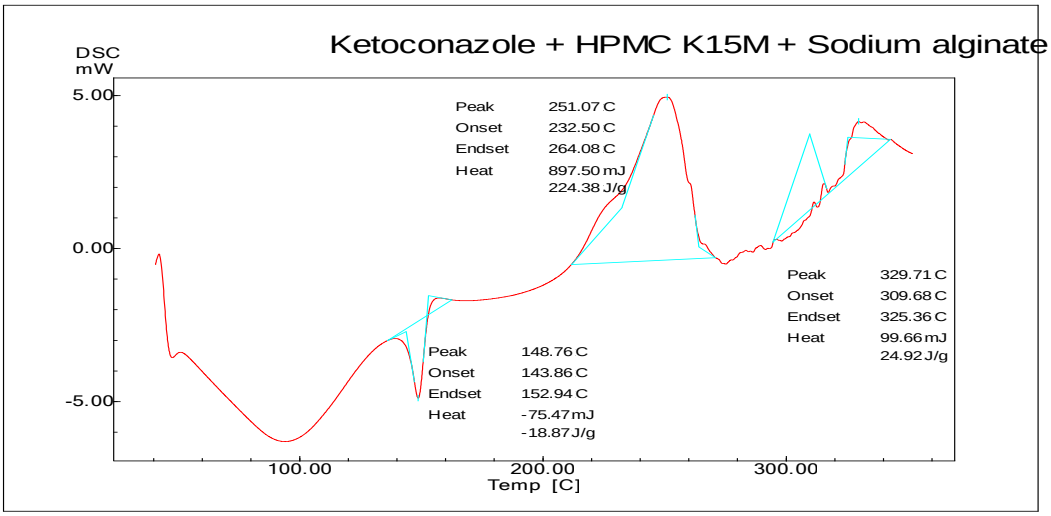


Fig (4): DSC Thermogram of a mixture of Ketoconazole +HPMC K15M + Sodium alginate

Standard calibration curve of Ketoconazole in 0.1N HCl buffer:

The standard calibration curve of Ketoconazole was obtained by plotting concentration v/s absorbance. The curve was found to is linear in the concentration range of 0-14 µg/ml at λmax 224.5 nm. The correlation coefficient (R^2) obtained was 0.994 and equation was y= 0.027 x + 0.059 Figure 5.

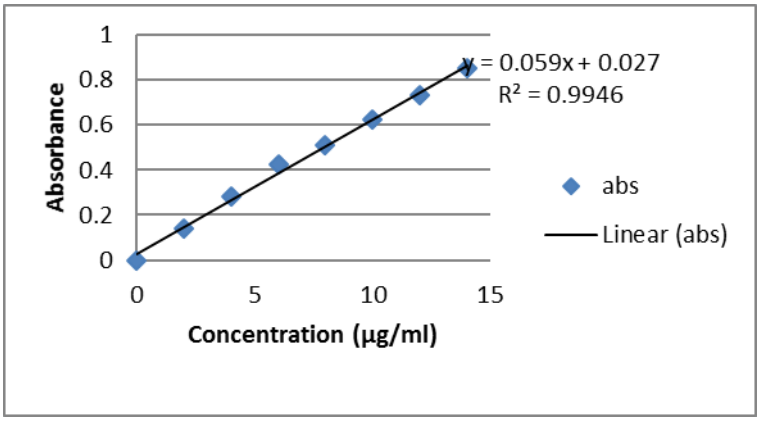


Fig. (5): Standard Calibration curve of Ketoconazole in pH 0.1N HCl buffer.

Design of Experiment

The various factors and levels of central composite design and other data generated by Central Composite design were showed in table 1 and 2.

Table (1): Factors and corresponding levels as per Central Composite Design

FACTORS	LEVELS		
	-1	**0**	**+1**
A: Sodium alginate	1.5 (%)	2.15 (%)	2.85 (%)
B: Olive Oil	7 (%)	10 (%)	12 (%)

Table (2): Amount of X_1 and X_2 generated by the software to be added in the 9 formulations

RUN	AMOUNT OF SODIUM ALGINATE (%)	AMOUNT OF OLIVE OIL (%)
1	2.15	10
2	1.5	7
3	2.85	7
4	1.5	12
5	2.85	12
6	2.85	10
7	2.15	7
8	2.15	12
9	1.5	10

Formulation of floating alginate beads:

Total 10 formulations were developed according to central composite design and composition of each was tabulated in Table 3.

Table (3): Composition of stomach-specific floating alginate beads of Ketoconazole

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (mg)	200	200	200	200	200	200	200	200	200
Sodium Alginate (%)	2.15	1.5	2.85	1.5	2.85	2.85	2.15	2.15	1.5
HPMC K15M (%)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Olive oil (%)	10	7	7	12	12	10	7	12	10
CaCl2 (%)	5	5	5	5	5	5	5	5	5

Evaluation of floating microbeads:

Floating alginate beads formed were spherical in shape, having a rough surface with pores. They have good micrometric parameters and physical parameters. The results obtained for flow properties of the powder such as bulk density, tapped density, Carr's compressibility index, Hausner's ratio, angle of repose, true density and % Porosity were tabulated in Table 4

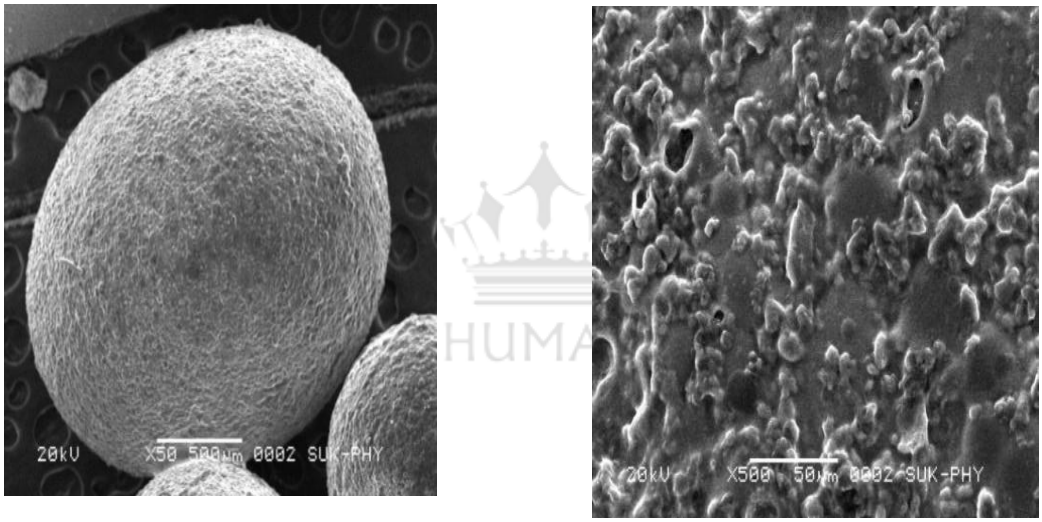
Table (4): Micrometric parameters F1-F9

F.Code	Bulk Density (g/cm3)	Tapped Density (g/cm3)	Hausner's ratio	Carr's Index(%)	The angle of Repose(θ)
F1	0.550±0.002	0.703±0.002	1.09±0.032	8.67±0.130	18.26°±0.040
F2	0.714±0.002	0.769±0.002	1.077±0.008	7.15± 0.1	23.27°± 0.045
F3	0.632±0.001	0.671±0.002	1.044±0.177	5.18± 0.036	15.10° ±0.030
F4	0.795±0.001	0.875±0.002	1.10±0.011	9.14± 0.035	18.26°± 0.015
F5	0.500±0.005	0.552±0.002	1.104±0.004	9.42 ±0.035	16.69°± 0.070
F6	0.611±0.003	0.690±0.002	1.12±0.030	11.42± 0.08	21.80°± 0.035
F7	0.622±0.002	0.700±0.002	1.05±0.026	5.42± 0.025	16.69°± 0.070
F8	0.664±0.016	0.708±0.001	1.06±0.025	6.21± 0.025	23.74° ±0.076
F9	0.685±0.016	0.774±0.002	1.12±0.030	11.49± 0.055	24.42° ±0.025

Physical parameters:

The formulated beads were evaluated for its physical parameters. Percentage yield of beads was found to be ranging from 80±0.001% to 90±0.002%. The particle size of sodium alginate

formulations was found to be increasing with the concentration of oil increases. The mean particle was found to be in the range of 0.96mm±0.011to 1.28mm±0.016. Entrapment efficiency was found to be in the range 63.28±0.009%-91.70±0.008. Results revealed that entrapment efficiency was found to increase with an increase in polymer concentration. Increase in concentration of polymers increases cross-linking of polymers which in turn prevents the diffusion of the drug. Swelling index tends to increase with an increase in polymer concentration due to increase in viscosity which in turn retards the release of the drug. The swelling index was found in the range of 7.22±1.76% to 7.63±1.23%. The density of beads is less than 1.004gcm^{-1}thus it remains buoyant for 8 hours. The morphological analysis of beads showed the nearly spherical shape and a rough external surface. The cross-sectional morphologies of floating beads were examined with SEM. The surface was found to be slightly porous which can be related to the concentration of oil. Figure 6.



a) Spherical in shape b) Rough surface with pores

Fig. (6): Scanning electron microscopy (SEM) study

Determination of buoyancy of beads:

All formulation of beads showed an instantaneous flotation with the floating lag time (8-16mins), good integrity and prolonged floating duration of more than 8 hours. Figure 7,8.

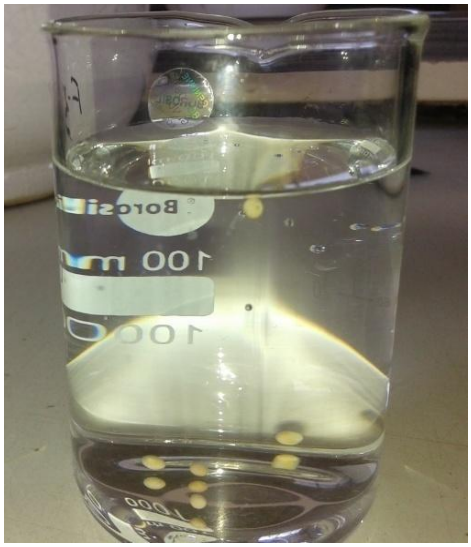


Fig. (7): Floating lag time

Fig. (8): Floating beads remained buoyant

Drug content:

Drug Content in the formulation was determined by using a UV-Spectrophotometric method using a standard calibration curve and results were found to be in the range of 83.6 ± 0.942 % - 95.27 ± 1.057 %. Results revealed that actual drug content was found to increase with an increase in polymer concentration.

***In vitro* drug release:**

The *in vitro* dissolution study for all the batches was performed for 480 minutes. At the end of the480minutesformulations showed drug release between 60.28 ± 0.006 to 77.95 ± 0.008%. Figure 9. Optimized F10 was compared with pure drug and F10 showed sustained release. Figure 10.

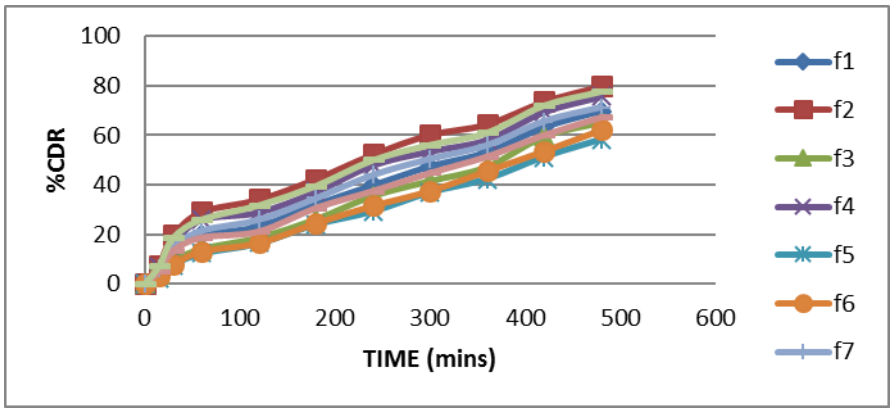


Fig. (9): *In Vitro* drug release profile of beads for formulation F1-F9 in 0.1N HCl Buffer

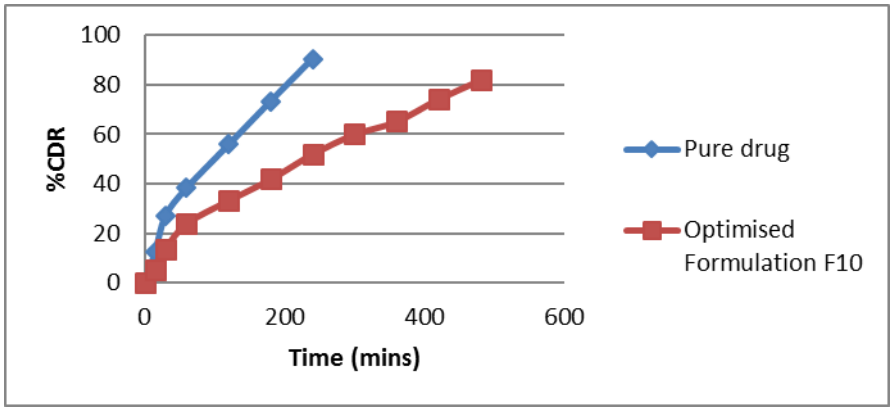


Fig. (10): *In Vitro* dissolution study of pure drug and optimized formulation F10

Release kinetics:

The release data were fitted to various kinetic models in order to calculate the release constant and regression coefficients (R^2). Among the models tested, the drug release profiles for formulations (F1, F7, F8) were fitted in Zero order model Formulations (F2, F4, and F9) were fitted in KrosmeyerPeppas model. Formulations (F3, F5, and F6) were fitted in HixCrowell model. The optimized formulation F10showsthepeppasasthe best fit model with non-fickian behaviour with an n value of 0.7008.

Optimization:

The regression analysis of the quadratic model fit revealed that particle size, floating lag time and % *in vitro* drug release were 97% correlated with active factors X_1, X_2. Based on the optimization results, ANOVA ($p< 0.05$) and desirability = 1, one solution was predicted by the software for independent variables X_1 and $X_2$with desired responses Y_1, Y_2, Y_3. Finally, a check-point batch F10 (optimized formulation) using optimized values is formulated to prove the validity of the evolved method. The results of evaluation parameters for optimized formulations F10 were found to be uniform and within the permissible limit. Table 5 to 9, Figure 11 and 12 countour plot and Figure 11 to 12 3D response surface.

Stability studies:

The optimized formulation F10 was selected for short-term stability studies at conditions 25^0C ±2.0^0C/ 60% RH% ±5.0% and accelerated stability studies were carried out at 40^0C±2.0^0C/ 75% RH% ±5.0%. For a period of 15 and 30 days, the beads were analysed for

Drug content, Density, Floating ability, Floating lag time and *in vitro* drug release at end of 480 minutes. The formulations did not show much variation in any of the parameters.

Table 5: Predicted solutions (optimized) by the Software: Factors and responses

Factors		**Response**		
X_1 (%)	**X_2(%)**	**Y_1(mm)**	**Y_2(mins)**	**Y_3(%)**
1%	8.5%	0.99	14.58	81.65

Table 6: Optimised formulation F10

Formulation Ingredients	**Quantities**
Ketoconazole	200 mg
Sodium alginate	1%
HPMC K15M	0.03%
Olive oil	8.5%
Calcium chloride	5%
Distilled water	q.s

Table 7: Evaluation parameters of optimized formulation F10

Evaluation Parameters	(Mean±sd)
Practical yield	81.27± 0.12%
Particle size	0.99mm ± 0.003
Entrapment efficiency	62.98% ± 0.04
Drug content	83.6%± 0.2
Swelling index	7.5± 0.1 %
In vitro drug release at 8 th hour	81.65± 0.01%
Kinetic release model	Peppas(r^2)= 0.9944

Table 8: *In vitro* Buoyancy study of F10

Floating lag time (mins)	Floating duration (hours)
14.58	>8

Table 9: Comparison between experimented values and predicted values of optimized formulation

Response variables	Experimented with values	Predicted values	% Error
Y_1 Particle size (mm)	0.99	1.00	-1
Y_2 Floating lag time (mins)	14.58	14.345	+1.64
Y_3 In vitro drug release at the end of 8 hours (%)	81.65	79.13	+ 3.18

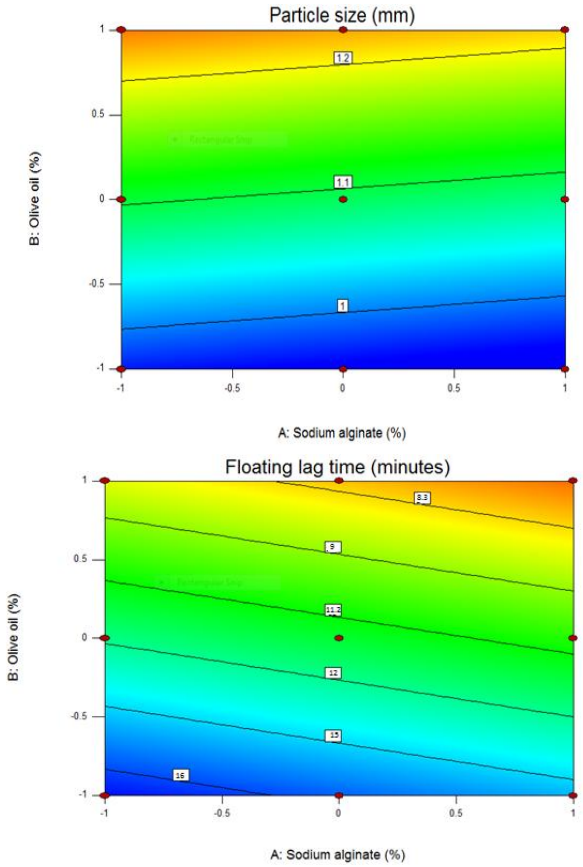


Fig. (11): Contour plots of response Y_1 AND Y_2

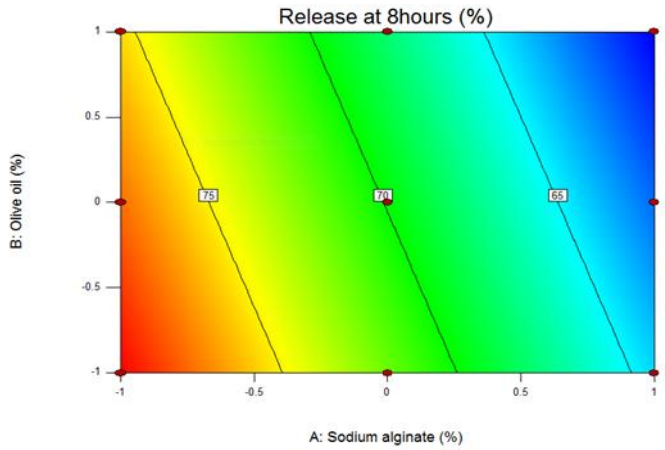


Fig. (12): Contour plot of response Y_3

DISCUSSIONS

FTIR spectra and DSC of the physical mixture revealed that the drug and the polymers used were compatible. The particle size of the gel beads increased as the concentration of oil used was increased. Entrapment efficiency, swelling index was found to be proportionally dependent on polymer concentration. Densities of all formulations were found to be less than 1.004g/cm^3 imparting the buoyancy of the beads more than 8 hours. The surface topography study revealed that the beads were spherical in shape with a slightly rough surface having small pores on the surface which may be responsible for drug release. The drug release decreased with the increase in polymer concentrations in beads. Formulations (F1, F7, F8) were fitted in Zero order model Formulations (F2, F4, and F9) were fitted in KrosmeyerPeppas model. Formulations (F3, F5, and F6) were fitted in HixCrowell model. Optimized formulation 10 was optimized based on practical yield, particle size, drug content, entrapment efficiency, floating lag time and *in vitro* drug release. KorsemeyerPeppas was found to be a best fit kinetic model. Formulation F10 was found to have a maximum release at the end of 8 hours. It was also subjected to stability studies as per ICH storage conditions. Stability studies revealed that formulation was stable for 1 month.

CONCLUSION

In the present study, Ketoconazole floating alginate beads were successfully prepared by ionotropic gelation method. The particle size of the gel beads increased as the concentration of oil used was increased. The density of all formulations was found to be less than 1.004g/cm^3 imparting the buoyancy of the beads for more than 8 hours. *In vitro* drug release

of the optimized formulation, F10 showed sustained release profile at the end of 8 hours. The optimized F10 was compared with pure drug and F10 showed sustained release. It was also subjected to stability studies as per ICH storage conditions. All practical result concludes that the floating bead of ketoconazole with alginate having a better-sustained action for patient compliance.

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